

SPECIMEN ADEQUACY AND EFFECT OF HUMAN PAPILLOMAVIRUS IN CERVICOVAGINAL SMEARS WITH SQUAMOUS INTRAEPITHELIAL ABNORMALITY

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Abstract-Cervicovaginal cytology is still a widely used cancer screening method that is well established in most countries. The present study aims at detecting and classifying squamous intraepithelial abnormalities and effects of certain infectious agents, particularly the cytopathic effects of human papillomavirus (HPV) in a series of cervicovaginal smears, according to the Bethesda system (TBS). The significance of specimen adequacy in revealing squamous intraepithelial abnormalities (SIEA) was also evaluated. Overall, 9066 smears were examined according to TBS. For interpretation of atypical squamous cell of undetermined significance (ASCUS), certain quality control (QC) guidelines were considered. While stressing on koilocytic atypia, the full spectrum of cytologic alterations due to HPV was investigated. Of all smears, 79% were satisfactory for evaluation and 15% were inadequately satisfactory due to absence of the endocervical/transformation zone (EC/TZ) component. The proportion of SIEA was significantly ($P < 0.05$) higher in smears having such a component. Of 246 cases with SIEA, 173 (1.90%) cases were classified as ASCUS, 46 (0.50%) as low grade squamous intraepithelial lesion (LSIL), and 27 (0.26%) as high grade squamous intraepithelial lesions (HSIL). Of the latter, 19 cases demonstrated HPV effects. ASCUS was the most frequent diagnosis for which QC criteria were fulfilled. On the basis of our study the adequacy of specimen is of paramount significance, with particular emphasis on the presence of EC/TZ component.

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Key words: Squamous epithelial abnormalities, ASCUS, LSIL, HSIL, HPV

INTRODUCTION

Since 1928 when Papanicolaou introduced smears of vaginal secretions as a screening method for cervical cancer, few reporting formats have been so accepted as the Bethesda system (TBS) in the standardization of such reports and to serve as a uniform descriptive terminology (1,2). However, in our country it may not be fully appreciated, resulting

in misinterpretation and poor pathologist-clinician relationship. In the present study, a series of cervicovaginal smears were evaluated for cytologic findings of squamous intraepithelial abnormalities (SIEA) according to TBS, and for infectious agents. Our main emphasis was on determination of the cytopathic effects of human papilloma virus (HPV) by cytomorphologic identification, which is currently the most convenient, rapid, economical and sensitive procedure available for the detection of HPV infection in the genital tract (3).

Finally, the significance of specimen adequacy (as the first element of TBS) in the detection of squamous intraepithelial abnormalities was evaluated.

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MATERIALS AND METHODS

Status of adequacy and frequency of SIEA were determined and classified as atypical squamous cell of undetermined significant (ASCUS), low grade squamous intraepithelial lesion (LSIL) and high grade squamous intraepithelial lesion (HSIL).

Overall, 9066 cervicovaginal smears submitted during three years (1998-2000) to the department of pathology, Mirza Koochak-Khan hospital, were thoroughly evaluated microscopically by a pathologist and a pathology assistant. The primary aim was precise cytologic detection and classification of SIEA, with adherence to the cytologic criteria and descriptive terminology of TBS (1,2).

Although the microscopic identification of koilocytes was of primary importance for cytologic detection of HPV cytopathic effects, a constellation of various cytoplasmic and nuclear changes attributed to HPV cytopathic effects were taken into account and actively searched for. These are thoroughly elaborated in cytologic literature (3).

The status of adequacy is also determined cytologically according to adequacy terminology and criteria stated by TBS (2) (Table 1). All criteria were strictly taken into account.

It has been postulated that smears containing the endocervical/transformation zone (EC/TZ) reveal SIEA more frequently. To demonstrate this and its statistical significance, all other smears without SIEA, were taken as control cases. These control cases, of course, were not unsatisfactory from the point of view of adequacy. Target cases (with SIEA) and control cases (without SIEA) were then segregated into two groups on the basis of presence or absence of the EC/TZ component. Chi-square test was used for statistical analysis.

RESULTS

As the first element of TBS, the status of specimen adequacy in our series have been as follows:

A. Satisfactory for evaluation: 7163 case (79 %)

B. Satisfactory for evaluation but limited by: 1687 cases (18.6%); 1360 (15%) of which were due to the

Table 1. Criteria for specimen adequacy

Satisfactory for evaluation

All of the following must be present:

- Appropriate labeling and identifying information relevant to clinical information (minimum: age and LMP).
- Adequate number of squamous cells (should cover more than 10% of slide surface)
- Adequate EC/TZ component (minimum: 2 clusters of 5 cells each, exception: marked atrophy).

Satisfactory for evaluation but limited by ...

One or more of the following must be present:

- Lack of clinical information
- Obscuring blood, inflammation, thick areas, poor fixation, air-drying, contaminants (obscuring 50- 75% of the cells)
- Absence of EC/TZ component. Note: Does not necessarily require a repeated smear.

Unsatisfactory for evaluation

One or more of the following must be present:

- Lack of patient identification
- Unacceptable slide (e.g., broken beyond repair)
- Scant squamous cells (covering less than 10% of slide surface)
- Obscuring blood, inflammation, thick areas, poor fixation, air-drying, contaminants (obscuring 75% or more of epithelial cells)

Abbreviations: LMP, last menstrual period; EC/TZ, endocervical/transformation zone.

absence of EC/TZ component, and the remaining due to other reasons.

C. Unsatisfactory for evaluation: 216 cases (2.38%).

The chi-square of the data presented in table 2 illustrating number of cases with or without EC/TZ component segregated according to the presence of SIEA is 6.25, indicating a *P* value of less than 0.05.

Table 2. Number of cases with or without SIEA segregated by presence or absence of EC/TZ components

SIEA	EC/TZ	
	+	-
+	221	25
-	6937	1335

Abbreviations: SIEA, squamous intraepithelial abnormalities; EC/TZ, endocervical / transformation zone

Table 3. Relative frequencies of SIEA and number that reveal HPV effect.

SIEA	No of cases	Percentage*	HPV effect	Percentage†
ASCUS	173	1.90	21	12.13
LSIL	46	0.50	19	41.30
HSIL	27	0.26	9	33.33
TOTAL	246	2.71	49	

Abbreviations: SIEA, squamous intraepithelial abnormalities; HPV, human papillomavirus; ASCUS, atypical squamous cell of undetermined significance; LSIL, low grade squamous intraepithelial lesion; HSIL, high grade squamous intraepithelial lesions.

*In all cases.

† In each group of SIEA.

Fungal agents, coccobacillary organisms and *Trichomonas vaginalis* were present in 8.66%, 6.91% and 1.20% of cases, respectively.

Table 3 indicates the relative frequencies of SIAE subtypes and the percentage of smears revealing cytopathic features of HPV infection in each group. Of 46 cases classified as LSIL, 39 were consistent with CINI, 12 cases of which were accompanied by cytopathic evidence of HPV infection. The remaining 7 were specifically ascribed to HPV effect. Nine out of 27 cases of HSIL revealed HPV effect.

Mean age of patients having smears diagnosed as LSIL and HSIL were 42.17 and 40 years, respectively. The percentage of fungal, coccobacillary and trichomonal agents in cases showing SIEA were 4.87%, 3.25% and 1.2%, respectively. Specifically, 3.70% of HSIL cases showed clear-cut trichomonal infection.

DISCUSSION

Cervicovaginal cytology is still a widely used cancer screening method that is well established in our country. To compare the frequencies of cytologic abnormalities of Iranian patients with those in the literature, adherence to terminologies and criteria stated by the Bethesda system (TBS) has been our primary goal.

As the first element of TBS, specimen adequacy is of paramount significance. Considering great

differences in reported rates of inadequately satisfactory smears represented in literature (2), we compare our obtained rate (15 %), with the figure reported (36%) in another Iranian article (4), indicating fairly satisfactory method of sampling in Mirza Koochak-Khan hospital. Speaking in terms of statistics, we succeeded to maintain that smears containing EC/TZ component have a significantly higher frequency of SIEA detected than those lacking such cells ($P<0.05$). This had been demonstrated in numerous cross-sectional foreign studies (5-7).

We have demonstrated the frequencies of different lesions (ASCUS, LSIL, HSIL), and most importantly the rate of HPV cytopathic effects in each group. In our series, the most frequent diagnosis of SIEA lesions was ASCUS, comprising 1.90% of all cases. Regarding quality control (QC) of the obtained ASCUS rate (1.9%), two different criteria are represented in literature (2). First, this rate has to be less than 5% of all smears, and second it must be 2-3 times that of squamous intraepithelial lesions (SIL) rate. In our cases ASCUS rate was obviously less than 5% and 2.37 times that of SIL rate. Thus, both ASCUS QC criteria were fulfilled, making our obtained rate acceptable.

Regarding SIL, the total frequency (0.76%) was fairly lower than the rate mentioned in major cytology references (8). However, it has to be taken into account that at the present time, the Iranian population is low-risk for HPV infection, compared with Western societies. Accordingly, the 1955 survey by Regan and coworkers, reporting a rate of 0.77% for dysplastic changes in cervicovaginal smears can serve as an appropriate base upon which we can make comparison (9).

We conclude, that for the detection of SIEA, the adequacy of specimen is of paramount significance, with particular emphasis on the presence of the EC/TZ component. Moreover, the ASCUS rate obtained in our department was acceptable because it fulfilled QC criteria.

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